

## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

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PHYSICO-CHEMICAL PROPERTY – MELTING POINT	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Testing was conducted as specified in Commission Directive 92/69/EEC
Test Type	Melting Point
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Melting point determination was carried out using the Capillary Method/Melting Temperature Devices with Liquid Bath, Method A1 of Commission Directive 92/69/EEC.
<b><u>Results</u></b>	The test material was determined to melt at 191°C with decomposition above 228°C.
<b><u>Data Quality</u></b>	Reliable without restrictions – Klimisch Code 1
<b><u>References</u></b>	OS114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>PHYSICO-CHEMICAL PROPERTY – OCTANOL/WATER PARTITION COEFFICIENT</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Testing was conducted according to method A8 specified in Commission Directive 92/69/EEC
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Octanol/water partition coefficient was determined by measuring the amount of test material (5 mg/L aqueous solution) that distributed into n-octanol (water-saturated) after 5 min of flask shaking at 22°C. Test material distributed into the n-octanol phase was measured using high performance liquid chromatography and UV detection.
<b><u>Results</u></b>	The octanol/water partition coefficient of the test material was determined to be $3.87 \times 10^{-4}$ at 22°C. $\text{Log}_{10}P_{ow} = -3.41$
<b><u>Data Quality</u></b>	Reliable without restrictions – Klimisch Code 1
<b><u>References</u></b>	OS114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>PHYSICO-CHEMICAL PROPERTY – SOLUBILITY</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Testing was conducted according to method A6 specified in Commission Directive 92/69/EEC
Test Type	Water solubility
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Water solubility was determined by measuring the amount of solid test article that distributed into distilled water after 17 hours of flask shaking at 20°C. The solubilized test material was measured using high performance liquid chromatography and UV detection.
<b><u>Results</u></b>	
	The water solubility of the test material was determined to be 761 g/l at 20°C.
<b><u>Data Quality</u></b>	
	Reliable without restrictions – Klimisch Code 1
<b><u>References</u></b>	
	OS114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
<b><u>Other:</u></b>	
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PHYSICO-CHEMICAL PROPERTY – HYDROLYSIS	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Potentiometric titration
Test Type	Hydrolysis
GLP (Y/N)	N
Year (Study Performed)	1978
Remarks field for test conditions	The hydrolysis of AMPS monomer sodium salt at pH=12 creates sodium acrylate and β, β-dimethyltaurine. The hydrolysis was done at 50°C, with 631 ppm 4-methoxyphenol and at 80°C with 506 ppm of 4-methoxyphenol. The hydrolysis was followed by potentiometric titration with perchloric acid in glacial acetic acid. Sodium acrylate, a strong base, and β, β-dimethyltaurine, a weak base were measured.
<b><u>Results</u></b>	At 50°C, there was only about 6% hydrolysis in 7 days. At 80°C, the half-life of the hydrolysis was about 5 days.
<b><u>Data Quality</u></b>	Reliable with restrictions – Klimisch Code 1
<b><u>References</u></b>	Unpublished confidential business information
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>PHYSICO-CHEMICAL PROPERTY – HYDROLYSIS</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Remarks	This substance is referred to as Sodium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Potentiometric titration
Test Type	Hydrolysis
GLP (Y/N)	N
Year (Study Performed)	1978
Remarks field for test conditions	AMPS monomer is a strong acid. The hydrolysis of AMPS monomer at pH=1 creates two weak acids, acrylic acid and β, β-dimethyltaurine. The hydrolysis was done at 50°C, with 631 ppm of 4-methoxyphenol to inhibit polymerization, and at 80°C with 506 ppm of 4-methoxyphenol. The hydrolysis was followed by the potentiometric titration of the solution with tetrabutylammonium hydroxide in t-butyl alcohol. Endpoints for both the strong acid, AMPS, and for the weak acids, acrylic acid and β, β-dimethyltaurine were observed. The degree of hydrolysis was calculated from the ratio of strong acid to weak acid.
<b><u>Results</u></b>	At 50°C, hydrolysis was negligible. At 80°C, the half-life of the hydrolysis was about 7 days.
<b><u>Data Quality</u></b>	Reliable with restrictions – Klimisch Code 1
<b><u>References</u></b>	Unpublished confidential business information
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PHYSICO-CHEMICAL PROPERTY – VAPOR PRESSURE	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	Testing was conducted according to method A4 specified in Commission Directive 92/69/EEC
Test Type	Vapor pressure
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Vapor pressure was determined using a vapor pressure microbalance with measurements being made at several temperatures. Linear regression analysis was used to calculate the vapor pressure.
<b><u>Results</u></b>	The vapor pressure of the test material was determined to be $7.4 \times 10^{-9}$ Pa at 25°C
<b><u>Data Quality</u></b>	Reliable without restrictions – Klimisch Code 1
<b><u>References</u></b>	OS114452: Determination of Vapor Pressure, SafePharm Laboratories LTD., 10/16/95.
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<b>AQUATIC TOXICITY – FISH</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	“Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians”, EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain/Supplier	Bluegill ( <i>Lepomis macrochirus</i> )
Analytical Monitoring	None
Exposure Period (unit)	96 hours
Statistical Methods	LC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	<p>Test Organisms: Source – In house laboratory supply (Lot # 83A13); Age- Juvenile; Length – 3.8 cm; Wet weight – 0.66 g; Loading rate – n/a; Pretreatment – none, fish were acclimated to the test conditions for 14 days prior to start of test</p> <p>Test System: The static acute test was conducted using nominal test concentrations of 130, 220, 360, 600 and 1000 mg/L. A stock solution (150 mg/ml) of the test substance was prepared and separately dispersed in each test vessel to yield the definitive test concentrations. The test was conducted glass exposure vessels that contained 15 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test solution was not aerated and maintained on a photoperiod of 16 hours light and 8 hours darkness. The fish were not fed during the test</p> <p>Dilution Water: Source – soft water reconstituted from deionized water; Hardness – Water adjusted to a hardness of 44 mg/L as CaCO<sub>3</sub>; Analysis – n/a; Water chemistry in test: DO (mg O<sub>2</sub>/L) – 5.5 to 9.2; pH – 7.5; specific conductance – 140 µmhos/cm</p> <p>Test Temperature (°C) – 22 ± 1</p> <p>Test Levels: Control, 130, 220, 360, 600 and 1000 mg/L</p>
<b><u>Results</u></b>	
	96-hour LC50 = >1000 mg/L based on nominal test concentrations
Remarks	Measured concentration: n/a

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	<p>Unit: mg/L</p> <p>96-hour LC0 = 1000 mg/L</p> <p>Statistical results: 96-hour LC50 = &gt;1000 mg/L. LC0 (0 – 96 hours) = 1000 mg/L</p> <p>Other:</p> <ul style="list-style-type: none"><li>• Effect concentrations based on nominal loading rates</li><li>• No mortality or sublethal effects were seen at the highest test concentration of 1000 mg/L.</li></ul>
<b><u>Conclusions</u></b>	96-hour LC50 = >1000 mg/L. 96-hour LC0 = 1000 mg/L
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<i>Acute Toxicity of OS # 48933F to Bluegill (Lepomis macrochirus). Bionomics study # - 043-0983-H77-100; Bionomics report # BW-83-11-1491. EG&amp;G Bionomics, November, 1983</i>
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>



## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>AQUATIC TOXICITY – INVERTEBRATE</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	“Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians”, EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	<i>Cladoceran</i> , <i>Daphnia magna</i>
Analytical Monitoring	N/a
Exposure Period (unit)	48 hours
Statistical methods	EC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>Test System: A stock solution (10 mg/ml) of the test substance was prepared in distilled water. An appropriate amount of the stock solution was then added to the appropriate amount of dilution water to yield 1000 ml. Each test solution was divided into 3 beakers to provide replicates containing 200 ml each. The test solution was not aerated during the exposure period. Daphnids were not fed during the test and maintained on a 16-hour light and 8-hour dark photoperiod.</p> <p>Dilution water: Well water was fortified according to the test protocol and filtered through a carbon filter and an Amberlite ZAD-7 resin column to remove any potential organic contaminants. The dilution water had a hardness of 160 - 180 mg/L as CaCO<sub>3</sub>. Analysis – n/a; Water chemistry in test: DO – &gt;60% saturation; pH – 7.9 – 8.3; specific conductance – 400 - 600 µmhos/cm</p> <p>Element: Immobilization/mortality</p> <p>Test Levels: Test concentrations included control, 130, 220, 360, 600 and 1000 mg/L. Fifteen daphnia were used per test group (5 per replicate).</p>
<b><u>Results</u></b>	
Remarks	<p>48-h EC<sub>50</sub> = &gt;1000 mg/L. 48-h EC<sub>0</sub> = 1000 mg/L</p> <p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Other:</p> <ul style="list-style-type: none"> <li>Effect concentrations based on nominal loading rates.</li> </ul>

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	<ul style="list-style-type: none"><li>No mortality or sublethal effects were seen at the highest test concentration of 1000 mg/L</li></ul>
<b><u>Conclusions</u></b>	48-h EC <sub>50</sub> = >1000 mg/L. 48-h EC <sub>0</sub> = 1000 mg/L
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<b><i>Acute Toxicity of OS # 48933F to Daphnia magna. Bionomics study # - 043-0983-H77-110; Bionomics report # BW-83-11-14901. EG&amp;G Bionomics, November, 1983</i></b>
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>ENVIRONMENTAL FATE – BIODEGRADATION</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	The Evaluation of the biodegradation of test materials using the semi-continuous activated sludge method. (40 CFR 795.3340)
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1986
Contact time (units)	44 days
Inoculum	Secondary activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	<p>Inoculum: Four liters of secondary activated sludge were collected from a domestic wastewater treatment plant. Raw sewage collected from the primary settling tank was used as the influent during acclimation and test periods.</p> <p>Concentration of test chemical: From day 1 to 8, 20-mg carbon/L influent was introduced into the test unit. This was reduced to 10-mg carbon/L from day 8. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium</p> <p>Test Setup: Aeration units consisted of glass vessels with cone shaped lower ends. The units contain 150 ml of mixed liquor when filled. A drain hole is located at the 50 ml level to facilitate sampling and removal of effluent. The acclimation process included aeration for 23 hours, settling of sludge, removing 100 ml of supernatant and resuming aeration to 500 mg/minute. The units were fed raw sewage daily till a clear supernatant liquor was obtained. At the end of the acclimation period, sludge from all units was pooled and mixed. Adequate amount was added to each unit to achieve a suspended solids concentration of 1500 mg/L for each unit. One-mg carbon/100 ml influent was added to the appropriate test units. Blank units did not receive any test material. The study was extended to 44 days to determine if acclimation and biodegradation will increase in this time frame.</p> <p>Sampling frequency: Samples were collected for DOC analysis on all workdays.</p> <p>Controls: Yes; blank unit was included.</p>

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	Analytical method: DOC analysis was conducted after sparging the samples with nitrogen prior to analysis to remove soluble carbon dioxide.  Method of calculating measured concentrations: N/A  Other:
<b><u>Results</u></b>	
Degradation % after time	<10% after 44 days.
Kinetic (for sample, positive and negative controls)	n/a
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<b><u>Conclusions</u></b>	The test substance showed a low biodegradation rate (<10%) in 28 days.
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<i>Evaluation of the biodegradation of OS 69793F using the modified SCAS method for Lubrizol Corporation. Ref: 86-0843-11; Hill Top Research Inc., Nov 12, 1986.</i>
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<b>AQUATIC TOXICITY – FISH</b>	
<b>Test Substance</b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b>Method</b>	
Method/Guideline followed	OECD 203 (1992)
Test Type	Static renewal (test media was renewed every 24 hours)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain/Supplier	Fathead minnow ( <i>Pimephales promelas</i> )
Analytical Monitoring	The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0, 24 and 96 hours. Analysis of the test solutions at 0, 24 and 96 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.
Exposure Period (unit)	96 hours
Statistical Methods	LC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947). Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. BACT Reviews, 11, pages 115-145.
Remarks field for test conditions	<p>Test Organisms: Source – Neil Hardy Aquatica, Carshalton, Surrey, UK; Age- Juvenile; Length – 3.8 cm; Wet weight – 0.54 g; Loading rate – 0.27 g/L; Pretreatment – none, fish were acclimated to the test conditions for 12 days prior to start of test</p> <p>Test System: The static acute test was conducted using nominal test concentrations of 200, 360, 640, 1120 and 2,000 mg/L. The test substance was separately dispersed in 20 litres of dechlorinated tap water to yeild the definitive test concentrations. The test was conducted glass exposure vessels that contained 20 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test vessels were convered to reduce evaporation and maintained on a photoperiod of 16 hours light and 8 hours darkness. The test vessels were aerated via narrow bore glass tubes. Test media were renewed daily. The fish were not fed during the test</p> <p>Dilution Water: Source – Dechlorinated tap water; Hardness – Water adjusted to a hardness of 100 mg/L as CaCO<sub>3</sub>; Analysis – Water was free of measurable quantities of pesticides; Water chemistry in test: DO (mg O<sub>2</sub>/L) – 8.2 to 8.5; pH – 7.4 to 7.7</p> <p>Test Temperature (°C) – 21</p> <p>Test Levels: Control, 200, 360, 640, 1120 and 2,000 mg/L</p>
<b>Results</b>	
	96-hour LC50 = 1400 mg/L based on nominal test concentrations
Remarks	Measured concentration: Analysis of the test solutions at 0, 24 and 96

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	hours showed the measured test concentrations to be near nominal as shown below: <table><tr><td></td><td>0-hour (% of nominal)</td><td>24-hour (% of nominal)</td><td>96-hour (% of nominal)</td></tr><tr><td>Control</td><td>&lt;LOQ</td><td>&lt;LOQ</td><td>&lt;LOQ</td></tr><tr><td>200 mg/L</td><td>100</td><td>99</td><td>103</td></tr><tr><td>360 mg/L</td><td>102</td><td>105</td><td>101</td></tr><tr><td>640 mg/L</td><td>101</td><td>99</td><td>102</td></tr><tr><td>1120 mg/L</td><td>101</td><td>100</td><td>102</td></tr><tr><td>2000 mg/L</td><td>103</td><td>102</td><td></td></tr></table> <p>Where LOQ = Limit of quantitation</p> <p>Unit: mg/L</p> <p>96-hour LC0 = 640 mg/L</p> <p>Statistical results: 72 and 96-hour LC50 = 1400 mg/L. 95% CL (1300 – 1600) mg/L.</p> <p>Other:</p> <ul style="list-style-type: none"><li>• Effect concentrations based on nominal loading rates</li><li>• No insoluble material was noted</li><li>• Sublethal effects including loss of equilibrium was seen at 96 hours in 1120 mg/L test concentration and after 3 hours in the 2000 mg/L test concentration.</li></ul>		0-hour (% of nominal)	24-hour (% of nominal)	96-hour (% of nominal)	Control	<LOQ	<LOQ	<LOQ	200 mg/L	100	99	103	360 mg/L	102	105	101	640 mg/L	101	99	102	1120 mg/L	101	100	102	2000 mg/L	103	102	
	0-hour (% of nominal)	24-hour (% of nominal)	96-hour (% of nominal)																										
Control	<LOQ	<LOQ	<LOQ																										
200 mg/L	100	99	103																										
360 mg/L	102	105	101																										
640 mg/L	101	99	102																										
1120 mg/L	101	100	102																										
2000 mg/L	103	102																											
<b><u>Conclusions</u></b>	72 and 96-hour LC50 = 1400 mg/L. 96-hour LC0 = 640 mg/L																												
<b><u>Data Quality</u></b>	<i>Reliable without restrictions– Klimisch Code 1</i>																												
<b><u>References</u></b>	<i>OS 114454: Acute toxicity to Fathead minnow (pimehales promelas). SPL Project Number: 525/049. SafePharm Laboratories Limited. May 2, 1996</i>																												
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AQUATIC TOXICITY – INVERTEBRATE											
Test Substance											
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt										
CAS #	58374-69-9										
Purity:	50% aqueous solution										
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category										
Method											
Method/Guideline followed	OECD #202 (1984)										
Test Type	Static acute toxicity test										
GLP (Y/N)	Y										
Year (Study Performed)	1996										
Species/Strain	Cladoceran, Daphnia magna										
Analytical Monitoring	The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0 and 48 hours. Analysis of the test solutions at 0 and 48 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.										
Exposure Period (unit)	48 hours										
Statistical methods	EC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947). Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. BACT Reviews, 11, pages 115-145.										
Remarks field for test conditions	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>Test System: The test material was prepared by direct dispersion in reconstituted water to give a 4000 mg/L stock solution from which dilutions were made in reconstituted water to prepare the test series. A 250-mL glass beaker that contained 200 mL of test solution was used per treatment. The test solution was not renewed during the exposure period. The test vessels were loosely covered to reduce entry of dust, etc. Daphnids were not fed during the test.</p> <p>Dilution water: Reconstituted water adjusted to a pH of 7.8 + 0.2. The dilution water had a hardness of 270 mg/L as CaCO3.</p> <p>Element: Immobilization/mortality</p> <p>Test Levels: Test concentrations included control, 36, 64, 112, 200, 360, 640, 1120, 2000 and 3600 mg/L. Ten daphnia were used per test group and each test included replicate vessels.</p>										
Results											
	48-h EC50 = 1200 mg/L. 48-h EC0 = 640 mg/.										
Remarks	<p>Measured concentration: Analysis of the test solutions at 0 and 48 hours showed the measured test concentrations to be near nominal as shown below:</p> <table><tr><td></td><td>0-hour (% of nominal)</td><td>24-hour (% of nominal)</td></tr><tr><td>Control</td><td>&lt;LOQ</td><td>&lt;LOQ</td></tr><tr><td>36 mg/L</td><td>93</td><td>95</td></tr></table>			0-hour (% of nominal)	24-hour (% of nominal)	Control	<LOQ	<LOQ	36 mg/L	93	95
	0-hour (% of nominal)	24-hour (% of nominal)									
Control	<LOQ	<LOQ									
36 mg/L	93	95									

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	112 mg/L	95	96
	360 mg/L	96	100
	1120 mg/L	97	98
	3600 mg/L	98	99
	Where LOQ = Limit of quantitation		
	Unit: mg/L		
	Other:		
	<ul style="list-style-type: none"><li>• Effect concentrations based on nominal loading rates.</li><li>• Control response was satisfactory (100% survival and no sublethal effects).</li></ul>		
<b><u>Conclusions</u></b>	48-h EC <sub>50</sub> = 1200 mg/L. 48-h EC <sub>0</sub> = 640 mg.		
<b><u>Data Quality</u></b>	<i>Reliable without restrictions– Klimisch Code 1</i>		
<b><u>References</u></b>	<b><i>OS 114454: Acute toxicity to Daphnia magna. SPL Project Number: 525/047. SafePharm Laboratories Limited. April 30, 1996</i></b>		
<b>Other:</b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>		



<b>ENVIRONMENTAL FATE – BIODEGRADATION</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	OECD 301B
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	28 days.
Inoculum	Return activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	<p>Inoculum: The sludge was aerated and stirred for 2 hrs in a flask, homogenized in a Waring blender at low speed for 2 minutes and let stand for 1 hour. The supernatant from the homogenized activated sludge was used as inoculum. The bacterial count in the supernatant was estimated using the Easicult dip-slide. The microbial level in the test mixture was 10,000 cells/mL</p> <p>Concentration of test chemical: A known concentration of test substance was added to the mineral medium, giving at least 30 mg ThOD per L medium. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium</p> <p>Test Setup: Seven flasks were used for the test substance, reference and controls. Each test vessel was connected to a series of 3 absorption bottles, each containing 100 mg of 0.0125 M (0.025N) barium hydroxide solution. The test was started by bubbling CO<sub>2</sub>-free air through the suspensions at a rate of 50 to 100 ml/min. The test vessels were covered with aluminum foil during the test period to prevent algal growth and photodegradation of test materials.</p> <p>Sampling frequency: During the first 10 days, the analysis of CO<sub>2</sub> was made every 2-3 days and then approximately every 5<sup>th</sup> day until the 28<sup>th</sup> day. CO<sub>2</sub> evolution from the test suspensions and inoculum blanks was followed in parallel.</p> <p>Controls: Yes; blank and positive controls (sodium benzoate), abiotic and toxicity checks were included. Sodium benzoate was used as the positive control</p> <p>Analytical method: For measurement of evolved CO<sub>2</sub>, the barium hydroxide absorber closest to the test vessel was disconnected and titrated with 0.1M HCl using phenolphthalein as the indicator.</p>

## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

	Method of calculating measured concentrations: N/A  Other:
<b><u>Results</u></b>	
Degradation % after time	3.3% after 28 days.
Kinetic (for sample, positive and negative controls)	Reference (sodium benzoate) – > 60% degradation in 4 days achieving a total of 93% in 28 days Abiotic degradation – 4.99% in 28 days, indicating insignificant physiochemical degradation of the test substance Toxicity check – Combined degradation of 48% for the reference and test substances, indicating the test substance was not toxic to the microbial populations in the inoculum.
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<b><u>Conclusions</u></b>	The test substance showed a low biodegradation rate (3.3%) in 28 days. The reference substance, sodium benzoate, reached a level of 93% in the same test period.
<b><u>Data Quality</u></b>	<i>Reliable without restrictions– Klimisch Code 1</i>
<b><u>References</u></b>	<i>OS 114454: Biodegradability study of test substance OS 114454 using the OECD CO2 evolution (Modified Sturm) test. Document NO. 65-95-0199-BC-001. Ricerca Inc. June 18, 1996.</i>
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>AQUATIC TOXICITY – ALGAE</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	OECD 201 (1984)
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	<b><i>Freshwater algae, Selenastrum capricornutum</i></b>
Element basis (# of cells/mL)	10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0 and 96 hours. Analysis of the test solutions at 0 and 96 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.
Statistical methods	Statistical analysis of the area under the growth curve data was carried out for the control and 2000 mg/L test groups using Students t-test.
Remarks field for test conditions	<p>Test Species: Cells taken from a log-growth phase in-house culture of <i>Selenastrum capricornutum</i> that was originally obtained from the Culture Centre for Algae and Protozoa (CCAP), Institute of Freshwater Ecology, Ferry House, Ambleside, Cambia.</p> <p>Test System: A limit test was conducted at a test concentration of 2000 mg/L. The test material was prepared by a direct dispersion in culture medium and volume adjusted to give a 4000 mg/L stock solution. An aliquot of the stock solution was mixed with 500 ml of algal suspension to give the test concentration of 2000 mg/L.</p> <p>Test Conditions: Six 250-ml conical flasks containing 100-mL of solution were prepared for the treatment group and 3 flasks were used for the control. At study initiation, the culture contained nominal cell density of ~10,000 cells/mL. The flasks were covered with aluminum foil and incubated at 24 ± 1°C, and constantly shaken at 100 rpm for 96 hours. Samples were taken at 0, 24, 48, 72 and 96 hours and the absorbance measured at 665 nm. Daily cell counts were made concurrently with a hemocytometer to confirm that the absorbance values were sufficiently well correlated with cell density values.</p> <p>Light: Cool-white fluorescent lights provided a light intensity of ~ 7000 lux under continuous illumination.</p> <p>Test temperature(°C) – 24 ± 1</p>

## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

	Dilution Water: Sterile enriched alga growth media adjusted to pH 8.0. pH ranged from 8.0 – 8.1 at 0-hour and 10.0 – 10.2 after 96 hours.												
<u>Results</u>	Test Levels: Control (3 replicates) and 2000 mg/L (6 replicates) Nominal concentrations: 72-h and 96-h EL <sub>50</sub> s = >2000 mg/L for both biomass and specific growth												
Remarks	<p>Measured concentration: Analysis of the test solutions at 0 and 96 hours showed the measured test concentrations to be near nominal as shown below:</p> <table><thead><tr><th></th><th>0-hour (% of nominal)</th><th>96-hour (% of nominal)</th></tr></thead><tbody><tr><td>Control</td><td>&lt;LOQ</td><td>&lt;LOQ</td></tr><tr><td>Treatment (R1 – R3)</td><td>96</td><td>100</td></tr><tr><td>Treatment (R4 – R6)</td><td>97</td><td>102</td></tr></tbody></table> <p>Where LOQ = Limit of quantitation</p> <p>Unit: mg/L</p> <p>There were statistically no significant differences (<math>P \geq 0.05</math>), between the control and 2000 mg/L test groups and therefore the no observed effect concentration (NOEC) is given as <math>\geq 2000</math> mg/L.</p> <p>Other:</p> <ul style="list-style-type: none"><li>• Effect concentrations based on nominal loading rates</li><li>• No insoluble material was noted</li><li>• Control response was satisfactory.</li></ul>		0-hour (% of nominal)	96-hour (% of nominal)	Control	<LOQ	<LOQ	Treatment (R1 – R3)	96	100	Treatment (R4 – R6)	97	102
	0-hour (% of nominal)	96-hour (% of nominal)											
Control	<LOQ	<LOQ											
Treatment (R1 – R3)	96	100											
Treatment (R4 – R6)	97	102											
<u>Conclusions</u>	No effects to algae growth or biomass was seen at the test concentration of 2000 mg/L.												
<u>Data Quality</u>	Reliable without restrictions– Klimisch Code 1												
<u>References</u>	<b>OS 114454: Algal Inhibition Test. SPL Project Number: 525/046. SafePharm Laboratories Limited. May 1, 1996</b>												
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>AQUATIC TOXICITY – FISH</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	“Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians”, EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain/Supplier	Bluegill ( <i>Lepomis macrochirus</i> )
Analytical Monitoring	None
Exposure Period (unit)	96 hours
Statistical Methods	LC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	<p>Test Organisms: Source – In house laboratory supply (Lot # 83A13); Age- Juvenile; Length – 3.8 cm; Wet weight – 0.66 g; Loading rate – n/a; Pretreatment – none, fish were acclimated to the test conditions for 14 days prior to start of test</p> <p>Test System: The static acute test was conducted using nominal test concentrations of 130, 220, 360, 600 and 1000 mg/L. A stock solution (150 mg/ml) of the test substance was prepared and separately dispersed in each test vessel to yield the definitive test concentrations. The test was conducted glass exposure vessels that contained 15 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test solution was not aerated and maintained on a photoperiod of 16 hours light and 8 hours darkness. The fish were not fed during the test</p> <p>Dilution Water: Source – soft water reconstituted from deionized water; Hardness – Water adjusted to a hardness of 44 mg/L as CaCO<sub>3</sub>; Analysis – n/a; Water chemistry in test: DO (mg O<sub>2</sub>/L) – 5.5 to 9.2; pH – 7.5; specific conductance – 140 µmhos/cm</p> <p>Test Temperature (°C) – 22 ± 1</p> <p>Test Levels: Control, 130, 220, 360, 600 and 1000 mg/L</p>
<b><u>Results</u></b>	
Remarks	<p>96-hour LC50 = 170 mg/L based on nominal test concentrations</p> <p>Measured concentration: n/a</p> <p>Unit: mg/L</p>

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**ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY**

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	<p>96-hour LC0 = 130 mg/L</p> <p>Statistical results: 72 and 96-hour LC50 = 170 mg/L. 95% CL (130 – 220) mg/L. LC0 (0 – 96 hours) = 130 mg/L</p> <p>Other:</p> <ul style="list-style-type: none"><li>• Effect concentrations based on nominal loading rates</li><li>• Sublethal effects including loss of equilibrium and rapid respiration was seen at 0 hours in the 600 and 1000 mg/L test concentration.</li></ul>
<b><u>Conclusions</u></b>	72 and 96-hour LC50 = 170 mg/L. 96-hour LC0 = 130 mg/L
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<i>Acute toxicity of OS # 26935F to Bluegill (Lepomis macrochirus). Toxicity test report submitted to Lubrizol Corporation. Bionomics Study # 043-0983-H76-100; Bionomics Report # BW-83-11-1492. EG&amp;G Bionomics, November, 1983.</i>
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>AQUATIC TOXICITY – INVERTEBRATE</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	“Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians”, EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain	<i>Cladoceran</i> , <i>Daphnia magna</i>
Analytical Monitoring	n/a
Exposure Period (unit)	48 hours
Statistical methods	EC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	<p>Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture.</p> <p>Test System: A stock solution (10 mg/ml) of the test substance was prepared in distilled water. An appropriate amount of the stock solution was then added to the appropriate amount of dilution water to yield 1000 ml. Each test solution was divided into 3 beakers to provide replicates containing 200 ml each. The test solution was not aerated during the exposure period. Daphnids were not fed during the test and maintained on a 16-hour light and 8-hour dark photoperiod.</p> <p>Dilution water: Well water was fortified according to the test protocol and filtered through a carbon filter and an Amberlite ZAD-7 resin column to remove any potential organic contaminants. The dilution water had a hardness of 160 – 180 mg/L as CaCO<sub>3</sub>. Analysis – n/a; Water chemistry in test: DO– &gt;60% saturation; pH – 7.9 – 8.3; specific conductance – 400 - 600 µmhos/cm</p> <p>Element: Immobilization/mortality</p> <p>Test Levels: Test concentrations included control, 78, 130, 220, 360, 600 and 1000 mg/L. Fifteen daphnia were used per test group (5 per replicate).</p>
<b><u>Results</u></b>	
	48-h EC <sub>50</sub> = 340 mg/L. 48-h EC <sub>0</sub> = 78 mg/.
Remarks	<p>Measured concentration: n/a</p> <p>Unit: mg/L</p> <p>Other:</p> <ul style="list-style-type: none"> <li>Effect concentrations based on nominal loading rates.</li> </ul>

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**ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY**

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	<ul style="list-style-type: none"><li>• Control response was satisfactory (100% survival and no sublethal effects).</li></ul>
<b><u>Conclusions</u></b>	48-h EC <sub>50</sub> = 340 mg/L. 95% Confidence limits = (280 – 430) mg/L. 48-h EC <sub>0</sub> = 78 mg/L
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<i>Acute toxicity of OS # 26935F to Daphnia magna. Toxicity test report submitted to Lubrizol Corporation. Bionomics Study # 043-0983-H76-110; Bionomics Report # BW-83-11-1489. EG&amp;G Bionomics, November, 1883.</i>
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<b>ENVIRONMENTAL FATE – BIODEGRADATION</b>	
<b><u>Test Substance</u></b>	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	
Method/Guideline followed	The Evaluation of the biodegradation of test materials using the semi-continuous activated sludge method. (40 CFR 795.3340)
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1986
Contact time (units)	44 days
Inoculum	Secondary activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	<p>Inoculum: Four liters of secondary activated sludge were collected from a domestic waste water treatment plant. Raw sewage collected from the primary settling tank was used as the influent during acclimation and test periods.</p> <p>Concentration of test chemical: From day 1 to 8, 20-mg carbon/L influent was introduced into the test unit. This was reduced to 10-mg carbon/L from day 8. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium</p> <p>Test Setup: Aeration units consisted of glass vessels with cone shaped lower ends. The units contain 150 ml of mixed liquor when filled. A drain hole is located at the 50 ml level to facilitate sampling and removal of effluent. The acclimation process included aeration for 23 hours, settling of sludge, removing 100 ml of supernatant and resuming aeration to 500 mg/minute. The units were fed raw sewage daily till a clear supernatant liquor was obtained. At the end of the acclimation period, sludge from all units was pooled and mixed. Adequate amount was added to each unit to achieve a suspended solids concentration of 1500 mg/L for each unit. One-mg carbon/100 ml influent was added to the appropriate test units. Blank units did not receive any test material. The study was extended to 44 days to determine if acclimation and biodegradation will increase in this time frame.</p> <p>Sampling frequency: Samples were collected for DOC analysis on all workdays.</p> <p>Controls: Yes; blank unit was included.</p>

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**ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY**

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	Analytical method: DOC analysis was conducted after sparging the samples with nitrogen prior to analysis to remove soluble carbon dioxide.  Method of calculating measured concentrations: N/A  Other:
<b><u>Results</u></b>	
Degradation % after time	<10% after 44 days.
Kinetic (for sample, positive and negative controls)	n/a
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<b><u>Conclusions</u></b>	The test substance showed a low biodegradation rate (<10%) in 28 days.
<b><u>Data Quality</u></b>	<i>Reliable with restrictions– Klimisch Code 2</i>
<b><u>References</u></b>	<i>Evaluation of the biodegradation of OS 61349E using the modified SCAS method for Lubrizol Corporation. Ref: 86-0844-11; Hill Top Research Inc., Nov 11, 1986.</i>
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>ACUTE TOXICITY – ORAL</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1981
Species:	Rat
Strain:	Sherman-Wistar
Route of administration:	Oral
Dose levels:	500, 1000, 2000, 4000 and 8000 mg/kg
Sex and number/group:	5 male rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation post-treatment
Control group:	No negative control group
<b><u>Result</u></b>	LD50 = 1830 mg/kg (Confidence Limits = 990-3390 mg/kg)
<b><u>Remark:</u></b>	Five groups of five male rats of the Sherman-Wistar strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. The sample was dosed as a 50% w/v suspension in water. The following dosages were administered: 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 4000 mg/kg and 8000 mg/kg. Following administration the animals were allowed food and water <i>ad libitum</i> for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. At the lowest dose tested, the animals were lethargic and ruffled after 2 hours. Their condition appeared essentially unchanged after 24 hours. The rats appeared normal within 48 hours. No unscheduled deaths occurred in this group. Animals receiving 1000 mg/kg were lethargic, ruffled and drooling after 1 hour. They were severely depressed after 3 hours. Deaths occurred in 2 animals after 4 hours. The surviving animals were semi-comatose after 24 hours. These animals remained moribund for the next 2-3 days during which time an additional death occurred. The two remaining animals in this group recovered after day 7-8. Animals receiving 2000 mg/kg were lethargic, ruffled and drooling after 30 minutes.

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**ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY**

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	They were severely depressed after 2 hours. Three animals died 3 hours after dosing. The surviving animals remained severely depressed or semi-comatose for several days before recovery at 7-9 days. Animals receiving 4000 mg/kg were severely depressed, ruffled and drooling 30 minutes after administration of the test article. Two animals died after 3 hours. The remaining animals were semi-comatose for the next 72 hours, with additional two animals expiring on days 3 and 4. The sole surviving animal recovered by day 9. All animals receiving 8000 mg/kg died within 15 minutes of test material administration. No remarkable gross pathological findings were observed. The LD50 was calculated employing the Thompson moving average method as modified by Weil.
<b><u>Reference</u></b>	Summary of results of acute toxicology studies: OS61349, Biosearch Inc., 4/28/81.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
<b><u>Other</u></b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>ACUTE TOXICITY – ORAL</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CASRN:	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1981
Species:	Rat
Strain:	Sprague-Dawley albino
Route of administration:	Oral
Dose levels:	1000, 2000, 4000, 8000 and 16000 mg/kg
Sex and number/group:	5 male rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
<b><u>Result</u></b>	LD50 > 16000 mg/kg
Remark:	Five groups of five male rats of the outbred Sprague-Dawley strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. The following dosages were administered: 1000 mg/kg, 2000 mg/kg, 4000 mg/kg, 8000 mg/kg and 16000 mg/kg. Following administration the animals were allowed food and water ad libitum for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. No unscheduled deaths were recorded and no unusual clinical or behavioral signs were observed in animals receiving dosages ranging from 1000-8000 mg/kg. Animals receiving 16000 mg/kg appeared ruffled and lethargic within 3-4 hours of test material administration. All animals appeared normal by day 5. No unscheduled deaths were recorded and gross examination revealed no pathological findings.
<b><u>Reference</u></b>	Summary of results of acute toxicology studies: OS48933E, Biosearch Inc., 12/17/81.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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<b>ACUTE TOXICITY – ORAL</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1993
Species:	Rat
Strain:	Sprague-Dawley albino
Route of administration:	Oral
Dose levels:	Limit study at 5000 mg/kg
Sex and number/group:	5 male and 5 female rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
<b><u>Result</u></b>	LD50 > 5000 mg/kg
<b><u>Remark</u></b>	<p>One group consisting of five male and five female rats of the Sprague-Dawley strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose of 5000 mg/kg by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. Following test material administration the animals were allowed food and water <i>ad libitum</i> for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. There were no unscheduled deaths during the study. Clinical findings were limited to abnormal defecation (soft stool, diarrhea) for three rats and a single occurrence of wet yellow urogenital staining on the day of dosing. One male had dried red material around the nose on day 2. There were no other clinical findings. There were no remarkable changes or differences in body weight during the study. One male had mottled lungs at the terminal necropsy. There were no test material-related gross necropsy findings for any examined tissues at terminal necropsy.</p>

## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

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<b><u>Reference</u></b>	Acute oral toxicity study of OS# 87613M in albino rats. WIL Research Laboratories, 12/15/93.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>ACUTE TOXICITY – DERMAL</b>	
<b><u>Test substance:</u></b>	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method:</u></b>	OECD 402
Type:	Acute dermal toxicity in rabbits
GLP:	Yes
Year:	1996
Species:	Rabbit
Strain:	New Zealand albino
Route of administration:	Single dose dermal application
Dose levels:	2000 mg/kg limit test
Sex and number/group:	5 male and 5 female rabbits per treatment group
Frequency of treatment:	Single 24 hour dermal application
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
<b><u>Result</u></b>	LD50 > 2000 mg/kg
Remarks:	One group consisting of five male and five female albino rabbits of the New Zealand strain weighing between 2000-2500 grams were employed in this study. Each animal was weighed and given a single dermal application (24-hour, semi-occluded exposure) of test article at a dose level of 2000 mg/kg. Following administration the animals were observed for 14-days for signs of toxicity and mortality. There were no unscheduled deaths during the study. Two rabbits had instances of soft stool on days 1 and 6, but this finding was determined not to be related to the test material. There were no other clinical findings. The test material induced very slight-to-slight erythema on all animals. Three rabbits had very slight edema. Desquamation was also observed on three rabbits. There were no other dermal findings. All edema subsided by day 2. All dermal irritation completely subsided by day 11 or earlier. There were no remarkable changes or differences in body weights. Upon terminal necropsy, two rabbits displayed accessory splenic tissue, a common congenital abnormality in New Zealand white rabbits. Dark red lungs were noted in two animals. There were no other remarkable findings.
<b>Reference</b>	Acute dermal toxicity study of OS# 114454 in albino rabbits. WIL Research Laboratories, 4/15/96.
<b>Data Quality</b>	Valid without restriction – (Klimisch Code 1)



**Other**

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<b>IN VITRO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 471
Type:	Bacterial reverse mutation assay
Year:	1984
GLP:	Yes
System of testing:	<i>S. typhimurium</i> strains: TA98, TA100, TA1535, TA1537, TA1538
Concentration:	0, 30, 100, 300, 1000 and 3000 microgram/plate
Metabolic activation:	With and without
<b><u>Results</u></b>	Non-mutagenic
Remarks:	<p>Concentrations for the mutagenicity assay were chosen on the basis of results from a range finding assay that showed the beginning of toxicity to be approximately 2000-6000 micrograms/plate. Thus, five concentrations of test material were prepared for use as described above. Each concentration was tested in duplicate in the referenced <i>Salmonella</i> strains, both in the presence and absence of a S9 rat liver metabolic activation system. Bacterial strains were checked the day of each assay to ensure that all five strains were sensitive to crystal violet (<i>rfa</i> mutation) and the TA98 and TA100 contained the R-factor (resistance to ampicillin). The maximum amount of test material used (3000 microgram/plate) was toxic to all strains in the assay. No mutagenic response was seen for stains TA98, TA100, TA1535 or TA1537 in either the activated or non-activated assays. Non-activated test material did produce a small increase in the number of revertants per plate, however, this increase was deemed to be the result of an unusually low number of revertant colonies on the corresponding negative control plates. The mutagenicity test was repeated to confirm the previous observations. The known mutagens used in the positive control experiments (2-anthramine, sodium azide, 2-nitrofluorene or 9-aminoacridine depending of the bacterial strain) produced significant increases in the frequency of revertants (approximately 40-fold over the DMSO-containing vehicle control). In conclusion, the test material did not induced significant increases in mutations in strains of <i>S. typhimurium</i>, either in the presence or absence of metabolic activation, up to toxicity limits. Thus, within the limitations of this assay, the test material was considered to be non-mutagenic.</p>

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<b><u>Reference</u></b>	Salmonella/microsome mutagenesis assay on OS# 61349B. Bioassay Systems Corp., 4/10/84.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
<b><u>Other</u></b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>IN VITRO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 471/472
Type:	Bacterial reverse mutation assay
Year:	1991
GLP:	Y
Type:	Bacterial reverse mutation assay
System of testing:	S. typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538, E. coli WP2
Concentration:	0, 15, 50, 150, 500, 1500 and 5000 micrograms/plate
Metabolic activation:	With and without
<b><u>Results</u></b>	Non-mutagenic
Remarks:	<p>Four strains of S. typhimurium and 1 strain of E. coli were used in the assays. The bacterial cultures were tested for genetic characterization (rfa mutation or ampicillin resistance) on the day of the assays. The test material was applied in an aqueous vehicle to bacteria at concentrations ranging from 15-5000 microgram/plate. Negative controls (sterile water or DMSO) or positive controls (2-aminoanthracene, 9-aminoacridine, sodium azide, N-ethyl-N'-nitro-N-nitrosoguanidine, 2-nitrofluorene depending on the bacterial strain and activation parameters) were used to verify the reliability of the assay. The metabolic activation system consisted of an S9 microsomal fraction obtained from the liver harvested from rats 5 days following a single intraperitoneal injection of Aroclor 1254. The mean number of revertants per plate and the standard deviation was calculated for each concentration and strain. A test result was considered positive if for any strain, a significant increase over the negative control in the number of revertants per plate was observed as concentration-dependent; or after a two-fold increase when the background was 50 revertants per plate or greater; or after a three-fold increase when the background was between 10 and 49 revertants per plate; or after a four-fold increase when the background was less than 10 revertants per plate. Each individual assay was performed in triplicate. The test substance was slightly toxic to all Salmonella strains at the highest dose used (5000 microgram/plate) both with and without metabolic activation. For all bacterial strains tested there was no significant increase in the number of revertants at any dose of test material when compared to the</p>

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**ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY**

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	corresponding negative solvent control. The entire assay was repeated and the results seen in the first experiment were confirmed. The positive and control solvent control for all experiments were within established historical ranges for each bacterial strain utilized. In conclusion, the test substance did not produce a significant increase in the number of revertants, with and without metabolic activation. Thus, at the concentrations tested and under the conditions of the assay, the test material was considered to be non-mutagenic.
<b><u>Reference</u></b>	Evaluation of OS61349H in a bacterial mutagenesis assay. A.D. Little Inc., 7/23/91.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
<b><u>Other</u></b>	<i>Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.</i>

<b>IN VITRO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 476
Type:	Mammalian Cell Gene Mutation Test
Year:	1991
GLP:	Y
System of testing:	Chinese hamster ovary cells
Concentrations:	Geometric increments from 130 – 4000 microgram/ml
Exposure period:	16 hours
Metabolic activation:	Without
Control and treatment:	The negative control was water vehicle and the positive control was ethylmethanesulfonate
<b><u>Results</u></b>	Non-mutagenic
Remarks:	<p>The objective of the study was to assess the ability of the test material to induce mutations in CHO at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. CHO cells obtained from the Oak Ridge National Laboratory were maintained in Ham's nutrient F12 medium supplemented with 10% fetal bovine serum, 50 U/ml penicillin, 50 microgram/ml streptomycin and 2 mM glutamine. These mycoplasma-free cells were grown at 37°C in 5% CO<sub>2</sub> and 95% relative humidity. For testing, cells were seeded at a density of 5 x 10<sup>5</sup> cells/100 mm tissue culture plate, and 24 hours later were treated in triplicate with either test material (400 mg/ml aqueous stock solution) or quality control preparations. The negative control was water vehicle and the positive control was ethylmethanesulfonate (235 microgram/ml). Experimental plates were cultured in the presence of non-activated test material ranging from 130-4000 microgram/ml for 16 hours. After that time period, medium containing the test material was removed, the cells detached under trypsin, washed and sub-cultured for 9-12 days to allow phenotypic expression. At the end of the phenotypic expression period, the cells were again sub-cultured in hypoxanthine-free Ham's F12 medium containing 10% fetal bovine serum and 10 uM thioguanine (five plates per test material concentration). These plates were incubated for 7-9 days, at which time the cells were fixed in methanol, stained in 5% Giemsa, dried and the mutant colonies counted. The HGPRT mutation frequency was calculated by dividing the total number of mutant colonies by the total number of cells seeded per</p>

	<p>culture (corrected for cloning efficiency). Preliminary range finding experiments revealed the test material to be toxic to CHO cells at concentrations of 2000 microgram/ml (50% cell loss) and 4000 microgram/ml (100% cell loss). The entire assay was repeated twice. At the concentrations tested, the assay material did not increase the frequency of mutant cells when compared to the negative vehicle control. In the first assay the mutation frequencies per 10<sup>6</sup> cells exposed to test material ranged from 13.2 to 78.3 and in the second assay the frequencies resulting from test material application ranged from 17.5 to 47.0 per 10<sup>6</sup> cells exposed. The mean mutation frequency for a comparable number of exposed cells exposed to water vehicle was 35.6 and 42.4, respectively. There was no statistically significant difference between the mutation frequencies at any dose of test material and the values obtained from the vehicle control (i.e., water). The positive control ethylmethanesulfonate produced mean mutation frequencies of 1308 and 241 per 10<sup>6</sup> cells exposed. The responses obtained from the negative and positive controls demonstrated that the system was capable of detecting mutagenic chemicals. Therefore, the experimental results indicate that the non-activated test material was non-mutagenic in CHO cells under the conditions of the assay.</p>
<b><u>Reference</u></b>	<p>CHO/HGRPT in vitro mammalian cell mutation assay without metabolic activation with OS 61349H. A.D. Little, Inc. 12/10/91.</p>
<b><u>Data Quality</u></b>	<p>Valid with restriction – (Klimisch Code 2) – The mutagenesis experiments performed with a metabolic activation system did not yield reliable results because the CHO cells did not grow. The assays were repeated, and the results were submitted in a separate report (see Mammalian Cell Gene Mutation Test below).</p>
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<b>IN VITRO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD
Type:	Mammalian Cell Gene Mutation Test
Year:	1992
GLP:	Y
System of testing:	Chinese hamster ovary cells
Concentrations:	Geometric increments from 10-5000 microgram/ml
Exposure period:	19 hours
Metabolic activation:	With and without
Control and treatment:	The negative control was water vehicle and the positive controls were ethylmethanesulfonate (235 microgram/ml) in the non-activated experiments and dimethylnitrosamine (100 microgram/ml) in the activated experiments
<b><u>Results</u></b>	Non-mutagenic
Remarks:	<p>The objective of the study was to assess the ability of the test material to induce mutations in CHO at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. CHO cells obtained from the Oak Ridge National Laboratory were maintained in Ham's nutrient F12 medium supplemented with 5% fetal bovine serum, 50 U/ml penicillin, 50 microgram/ml streptomycin and 2 mM glutamine. These Mycoplasma-free cells were grown at 37°C in 5% CO<sub>2</sub> and 90% relative humidity. For testing, cells were seeded at a density of <math>1 \times 10^6</math> cells/100 mm tissue culture plate, and 24 hours later were treated in duplicate with either test material (400 mg/ml aqueous stock solution) or quality control preparations. CHO cells were assayed in the absence and in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver homogenate supplemented with cofactors (S9). The negative control was water vehicle and the positive controls were ethylmethanesulfonate (235 microgram/ml) in the non-activated experiments and dimethylnitrosamine (100 microgram/ml) in the activated experiments. Assay plates were cultured in the presence of activated and non-activated test material (500 mg/ml aqueous stock solution) ranging from 10-5000 microgram/ml for 19 hours. After that time period, medium containing the test material was removed, the cells detached under trypsin, washed and sub-cultured for 7 days to allow phenotypic expression. At the end of the phenotypic expression period, the cells were again sub-cultured in</p>



	<p>hypoxanthine-free Ham's F12 medium containing 5% fetal bovine serum and 10 microM thioguanine (five plates per test material concentration). These plates were incubated for 8 days, at which time the cells were fixed in ethyl alcohol, stained in a dilute solution of crystal violet, dried and the mutant colonies counted. The HGPRT mutation frequency was calculated by dividing the total number of mutant colonies by the total number of cells seeded per culture (corrected for cloning efficiency). A test article was considered to be a mutagen if it; a) exhibited a dose-dependent increase in the average mutant frequencies (least-squares method of linear regression analysis; <math>p &lt; 0.05</math>), with at least one concentration producing a statistically significant increase in average mutant frequency that was greater than or equal to three times the average mutant frequency of the pooled concurrent negative controls, or b) represented a net increase of 20 mutants per <math>10^6</math> clonable cells. Preliminary range finding experiments revealed the test material to be toxic to CHO cells at concentrations <math>&gt; 3000</math> microgram/ml with and without metabolic activation. At the concentrations tested, the assay material did not increase the frequency of mutant cells when compared to the negative vehicle control. In the first experiment, the mutant frequencies of the negative control cultures ranged from 10.7 to 17.3 (mean 14.2, s.d. 6.9) survivors per <math>10^6</math> clonable cells. The cultures treated the test material (with and without metabolic activation) ranged from 4.6 to 33.0 mutants per <math>10^6</math> clonable cells. There were no statistically significant or dose-dependent increases in the average mutant frequencies of the cultures treated with either activated or non-activated test material. The test material was re-evaluated in a confirmatory assay under identical conditions. In these experiments, the average mutant frequencies of the negative cultures ranged from 4.5 to 33.9 (mean 19.2, s.d. 15.8) mutants per <math>10^6</math> clonable cells while those of the cells treated with activated or non-activated test material ranged from <math>&lt;2.5</math> to 29.1 mutant per <math>10^6</math> clonable cells. Again, there were no statistically significant or dose-dependent increases in the average mutant frequencies of the cultures treated with either activated or non-activated test material. The reliability of the assay for detecting mutagens was demonstrated by the observation that average mutant frequencies for the activated and non-activated positive controls were 334.5 and 275.0 survivors per <math>10^6</math> clonable cells. These results indicate that the test material, whether activated or non-activated, was non-mutagenic in CHO cells under the conditions of the assay.</p>
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<b><u>Reference</u></b>	CHO/HPRT Mammalian Cell Forward Mutation Assay on OS 61349J. Pharmakon Research International, Inc., 2/7/92
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>IN VITRO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 473
Type:	Mammalian cytogenetic test
Year:	1991
GLP:	Y
System of testing:	Chinese hamster ovary cells
Concentrations:	6 geometric increments ranging from 0 to approximately 6000 microgram/ml (dependent on exposure period and activation treatments)
Exposure period:	10 and 20 hours
Metabolic activation:	With and without
Controls and treatment:	Water vehicle as a negative control, and mitomycin C or cyclophosphamide as a positive controls
<b><u>Results</u></b>	Clastogenic after metabolic activation

Non-Activated				Activated			
Time (hrs)	Treatment	Dose (ug/ml)	% cells with aberrations	Time (hrs)	Treatment	Dose (ug/ml)	% cells with aberrations
10	Water		4.5	10	Water		2.7
	OS61349H	510	5.5		OS61349H	177	5.6
		1530	3.0			539	7.7*
		5100	7.0			1790	5.5
	Mitomycin C	0.3	36.0			3530	10.5*
20	Water		2			5890	NR
	OS61349H	344	2		Cyclophosphamide	50	46.2
		1150	1.5	10 (repeat)	Water		4.0
		3440	2		OS61349H	151	3.0
		5730	NR			505	6.3
	Mitomycin C	0.3	76			1510	6.0
						3010	10.3*
						5010	4.7
					Cyclophosphamide	50	40.0
				20	Water		4.5
						189	5.5
						630	1.5
* = p < 0.05 compared to negative control						1890	6.5
NR = Aberrations not scored due to cytotoxicity						6300	51.0* CT
CT = evidence of cytotoxicity					Cyclophosphamide	50	96
				20 (repeat)	Water		1.5
						1010	4.5
						2010	3.5
						3010	3.5
						4010	1.0
						5010	6.0*
					Cyclophosphamide	50	68

Table 1

Remarks:	<p>Chinese hamster ovary (CHO) cells (Merck Institute for Therapeutic Research) were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 50 units/ml penicillin, 50 microgram/ml streptomycin, 2mM glutamine, buffered with NaHCO<sub>3</sub>, and incubated under 95% relative humidity at 37°C and 5% CO<sub>2</sub>. The cells were determined to be free from mycoplasma contamination. Non-activated assay: CHO cells were seeded at a density of <math>5 \times 10^5</math> in 25 cm<sup>2</sup> culture flasks. The following day, the cells were exposed to media containing the test material (50% aqueous solution) at approximately 153, 306, 510, 1020, 1530, 3060 or 5100 microgram/ml for a duration of either 10 or 20 hours. Separate flasks were prepared containing either water vehicle as a negative control, or 0.3 microgram/ml mitomycin C as a positive control. All test sample concentrations and controls were tested in duplicate flasks. Two hours prior to harvest, vinblastine was added to 0.26 microgram/ml to arrest the cells in metaphase. At the end of this period, metaphase cells were collected with trypsin and concentrated by centrifugation. The cells were then washed in PBS, lysed in hypotonic KCL and sodium citrate and fixed in methanol:acetic acid (3:1). Drops of each lysate were dried on glass slides and stained with 5% Giemsa. Activated assay: CHO cells prepared similarly were exposure to test or control material for two hours in the presence of S9 microsomal fractions were prepared from the liver harvested from rats treated 5 days earlier with a single injection of Aroclor 1254 (500 mg/kg). In the activated experiments, cyclophosphamide at 50 microgram/ml was used as a positive control. Following this exposure, the cells were washed, re-incubated to complete the 10 or 20 hour time period and treated as described above. Mitotic index was determined on a minimum of 500 metaphase cells exposed to test material. At least 100 metaphase cells from a minimum of three selected test sample concentrations were analyzed for chromosomal aberrations. The data was analyzed using the statistical methods described by Margolin et al., Environ. Mutagen 8:183,204, 1986. Non-activated results (Table 3): The test material was toxic to CHO cells at 5730 microgram/ml in the 20-hour non-activated assay, whereas in the 10-hour assay, no toxicity was observed. None of the test material concentrations evaluated (344-2440 microgram/ml) caused a statistically significant increase in the number of chromosomal aberrations compared to vehicle controls after either exposure period. Activated results (Table 3): The test material was also assayed 10 and 20 hours after microsomal</p>
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	activation. Toxicity was observed at the highest concentrations tested (approximately 6000 microgram/ml) after 10 and 20 hours. The activated test material produced statistically significant chromosomal damage in CHO cells after 10 hours at approximately 3000 micrograms/ml and after 20 hours at concentrations of 5010 and 6300 microgram/ml. Based upon these results, the Study Director concluded that the activated test material was clastogenic in Chinese hamster ovary cell culture. However, this finding is confounded by the absence of a dose-response effect, lack of a time-response effect, lack of reproducibility between repeat experiments and the presence of extensive cytotoxic damage concurrent with observed chromosomal damage for one scored timepoint.
<b><u>Reference</u></b>	The Evaluation of OS 61349H in the In Vitro Chromosomal Aberration Assay. A.D. Little, Inc. 11/4/91.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

IN VIVO GENETIC TOXICITY	
<b><u>Test substance:</u></b>	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 475
Type:	Bone marrow cytogenetics test
Year:	1992
GLP:	Yes
Species:	Rat
Strain:	Sprague-Dawley
Sex:	5 male and 5 female rats per treatment group
Route:	Oral gavage
Exposure period:	6, 18 and 24 hours
Doses:	150, 500 and 1500 mg/kg
Controls and treatment:	Concurrent vehicle negative control (water), positive control animals treated with cyclophosphamide
<b><u>Results:</u></b>	Non-clastogenic

Male				Female			
Time (hrs)	Treatment	Dose (mg/kg)	% cells with aberrations	Time (hrs)	Treatment	Dose (mg/kg)	% cells with aberrations
6	Vehicle		3.2	6	Vehicle		2.0
	OS61349J	150	0.8		OS61349J	150	2.0
		500	1.6			500	0.8
		1500	1.2			1500	1.2
18	Vehicle		0.4	18	Vehicle		0.4
	OS61349J	150	0.4		OS61349J	150	1.2
		500	0.4			500	0.8
		1500	0.8			1500	1.2
24	Cyclophosphamide	30	18.8	24	Cyclophosphamide	30	19.2
	Vehicle		0.8		Vehicle		1.6
	OS61349J	150	0.0		OS61349J	150	1.2
		500	0.8			500	2.4
		1500	0.8			1500	0.8

### **Remarks**

The test material solution was administered to 8-9 week old test animals by oral gavage at a single dose of 150, 500 or 1500 mg/kg using plastic syringes and stainless steel intubation needles. The animals were not fasted prior to dosing. Six males and six females were dosed per group to ensure that acceptable metaphase cells could be obtained from 5 animals per group. Fifty metaphase cells from each of 5 animals per test condition were analyzed when possible. The total number of cells analyzed per dose at each exposure period equaled 250. Exposure time included 6, 18 and 24 hours. Clinical signs observed in the animals

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	<p>dosed at 1500 mg/kg included increased respiratory rate (50%), watery feces (28%), soft feces (17%), and wheezing (1%). Mean body weights of the dose groups were not significantly different from the vehicle control group at any time point. As is illustrated in Table 1 above, the test article did not produce statistically significant increases (<math>p &lt; 0.05</math>) in the percentage of cells with aberrations at any dose, time period or for either sex, compared to control values. Positive control animals treated with cyclophosphamide (30 mg/kg) demonstrated an increase in the frequency of damaged cells which was statistically significant at the study threshold of <math>p &lt; 0.05</math>. Thus, the positive and negative controls demonstrated the reliability of the assay to detect chromosomal aberrations. The percentage of cells with chromosomal aberration were analyzed by the statistical methods described by Margolin et al., Environmental Mutagenesis, Volume 8, 1986. These results indicate that the test material was non-clastogenic in rat bone marrow cells under the conditions of the assay.</p>
<b><u>Reference</u></b>	Evaluation of OS61348J in the in vivo chromosomal aberration assay. A.D. Little Inc., 1/7/92
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

<b>IN VIVO GENETIC TOXICITY</b>	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 474
Type:	Micronucleus assay
Year:	1996
GLP:	Y
Species:	Mouse
Strain:	Crl:CD-1 (ICR)BR
Sex:	Five male and five female mice/dose/time period
Route:	Intraperitoneal
Exposure period:	24, 48 and 72 hours
Doses:	175, 875 and 1750 mg/kg
Controls and treatment:	Concurrent vehicle negative control (water), positive control animals treated with cyclophosphamide
<b><u>Results</u></b>	The test material was considered to be non-clastogenic in the mouse micronucleus test, under the conditions and according to the criteria of the test protocol.

Male				Female			
Time (hrs)	Treatment	Dose (mg/kg)	% MPCE Frequencies	Time (hrs)	Treatment	Dose (mg/kg)	% MPCE Frequencies
24	Vehicle		0.03	24	Vehicle		0.03
	OS114454	175	0.07		OS114454	175	0.08
		875	0.03			875	0.04
		1750	0.02			1750	0.05
	Cyclophosphamide	60	2.24		Cyclophosphamide	60	1.39
48	Vehicle		0.04	48	Vehicle		0.05
	OS114454	175	0.01		OS114454	175	0.08
		875	0.04			875	0.03
		1750	0.03			1750	0.07
72	Vehicle		0.03	72	Vehicle		0.05
	OS114454	175	0.04		OS114454	175	0.05
		875	0.07			875	0.02
		1750	0.07			1750	0.03

Remarks	The test material solution was administered to ~ 7 week old test animals by intraperitoneal injection at a single dose of 175, 875 or 1750 mg/kg at a constant volume of 10 ml/kg. Nine groups of mice were used to evaluate the induction of micronuclei (five male and five female mice/dose/time period). Negative control (deionized water vehicle) and positive control (cyclophosphamide; 60 mg/kg) were administered concurrently for sacrifice at all three time
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	<p>points. Groups were sacrificed after 24, 48 and 72 hours, with the exception of the control groups, which were sacrificed at 24 hours. Bone marrow slides were prepared, stained and scored for the number of micronucleated polychromatic erythrocytes (MPCE) in a total of 2000 polychromatic erythrocytes (PCE) per mouse. The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE ratio) was also determined for each mouse as an index of toxicity. The frequency of MPCE in each test group was compared to its respective negative control group using a one-tailed Student's t-test with a post hoc Cochran-Armitage test for possible dosing trends. Analysis of the by-sex data indicated that the test material did not induce any statistically significant or dose-dependent increases in MPCE frequencies at any harvest time evaluated, as compared to concurrent negative controls (Table 2). However, a single isolated increase in the frequency of MPCEs (combined-sex) was observed in mice treated with the test material at a dose of 175 mg/kg and harvested at 24 hours (<math>p &lt; 0.05</math>). Since this increase was within the historical negative control range as well as the acceptable negative control values for this assay, this isolated increase in MPCE frequency was considered a statistical aberration due to a random fluctuation of the spontaneous MPCE frequency. In addition, the test material did not induce any statistically significant depressions in PCE/NCE ratios compared to concurrent negative controls. MPCE frequencies for all negative control groups were within acceptable negative ranges, and the cyclophosphamide positive control groups produced statistically significant increases in MPCE frequencies (combined- and by-sex; <math>p &lt; 0.01</math>). Cyclophosphamide-treated animals experienced significant depressions in PCE/NCE ratios (<math>p &lt; 0.05</math>). In conclusion, the test material was considered to be non-clastogenic in the mouse micronucleus test, under the conditions and according to the criteria of the test protocol.</p>
<b><u>Reference</u></b>	In vivo micronucleus test with OS# 114454 in mouse bone marrow erythropoietic cells. Pharmakon USA, 7/3/96.
<b><u>Data Quality</u></b>	Valid without restriction – (Klimisch Code 1)
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## ROBUST SUMMARIES FOR TOXICITY TESTING ON AMPS® CATEGORY

REPEAT DOSE TOXICITY	
<b><u>Test substance</u></b>	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
<b>CASRN:</b>	58374-69-9
<b>Purity:</b>	58.4% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 407
Type:	Repeated dose oral toxicity in rats
Year:	1995
GLP:	Y
Species:	Rat
Strain:	Sprague-Dawley [CrI:CD (SD)BR] – 44 days old at initiation of dosing
Sex:	10 male and 10 female rats per group
Route of Administration:	Oral gavage
Exposure Period:	28 days
Frequency of Treatment:	7 days/week
Post-exposure observation period:	14 days
Dose:	0, 50, 150, 400 or 1000 mg/kg
Control group:	Concurrent negative control group treated with water
NOEL:	1000 mg/kg/day
<b><u>Results</u></b>	<p>Analysis of the test material confirmed the homogeneity and stability of the sample preparations. All animals survived throughout the study. With the exception of a single high dose male, there were no remarkable clinical observations resulting from oral administration of the test material. One male receiving 1000 mg/kg/day exhibited a number of adverse signs (lethargy, emaciation, watery stool, yellow ano-genital staining, and decreased fecal volume) during the first week of the study but recovered by the end of week 2. No unusual postmortem findings were seen in this particular animal at study termination. Mean body weights and body weight gains for the 1000 gm/kg/day male group were slightly lower than control values at week 1 (not statistically significant). Values for the control and treated groups of males were comparable from weeks 2 through 4. Body weight values for control and treated females were comparable throughout the study. Food consumption in the male 1000 mg/kg/day group was transiently lower during week 1. Values in subsequent weeks were similar to, or higher than, control values. No effect on food consumption was seen in with any other dose either in the male or female groups. No effects of test material administration on</p>

	<p>hematology values were evident after four weeks of treatment or after the 2-week treatment-free recovery period (including but not limited to hemoglobin concentration, hematocrit, RBC count, platelet count, MCV, MCH, MCHC, white cell differential, PTT, aPTT, RBC morphology). No effects of test material administration on clinical chemistry indices were evident during the treatment or recovery phases of the study (including but not limited to electrolytes, liver enzymes, plasma proteins, serum lipids, glucose, creatinine and BUN). Urinalyses were unremarkable (i.e., protein, glucose, ketone, occults blood, pH, bilirubin, urobilinogen, appearance, specific gravity). No effects of test material administration were observed on organ weights (i.e., adrenal glands, brain, kidneys, liver, ovaries, testes) for any dose, sex or phase of the study. None of the macroscopic findings in rats sacrificed at the end of the treatment and post-treatment recovery periods were considered to be related to treatment with the test material. No test material-related changes in microscopic findings on 29 various organs or tissues were observed for any group. Mean values of all dose groups were compared to the mean value for the control group at each time interval. Statistical evaluation was made by the appropriate one way analysis of variance technique followed by a post-hoc comparison procedure (e.g., Dunnett's test or Kruskal-Wallis test). Oral gavage administration of the test material to rats at doses of 50, 150, 400 and 1000 mg/kg/day produced no significant toxicity at any dose level. Therefore, under the conditions of this study, the No Observed Effect Level (NOEL) was determined to be 1000 mg/kg/day.</p>
<b><u>Reference</u></b>	<p>A 4-week Toxicity Study of OS 14454 in the Rat via Oral Gavage Administration Followed by a 2-week Recovery Period. Huntingdon Life Sciences, 7/9/96.</p>
<b><u>Data Quality</u></b>	<p>Valid without restriction – (Klimisch Code 1)</p>
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<b>REPRODUCTION TOXICITY</b>	
<b>Test substance:</b>	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
<b>CASRN:</b>	58374-69-9
<b>Purity:</b>	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS® category
<b><u>Method</u></b>	OECD 421
Type:	Repeated dose oral reproductive/developmental toxicity screen in rats
Year:	2000
GLP:	Y
Species:	Rat
Strain:	Sprague-Dawley [CrI:CD (SD)BR] - Approximately nine weeks of age at initiation of the study
Sex:	12 male and 12 female rats per group
Route of Administration:	Oral gavage
Frequency of Treatment:	7 days/week
Dose:	0, 100, 500, or 1000 mg/kg
Control group:	Concurrent with water vehicle
Pre-mating exposure period for males:	14 days
Pre-mating exposure period for females:	14 days
Reproductive and Developmental NOEL:	F0 generation NOEL = 1000 mg/kg/day F1 generation NOEL = 1000 mg/kg/day
<b><u>Remarks</u></b>	Chemical analyses demonstrated that the test article was homogenous and stable at the concentrations used in the study. Dosing preparations were administered by oral gavage, as a single daily dose to F0 males and females beginning two weeks prior to mating. The F0 males were dosed for approximately seven weeks, including the two weeks prior to mating, during mating and through post-mating. The F0 females were dosed throughout the study, including the two weeks prior to mating, during mating, during gestation, and following parturition. Following 14 days of treatment with test material or vehicle control, each F0 female was cohabitated with a single F0 male randomly selected from the same treatment group. The day of confirmed copulation was designated as day 0 of gestation and the female was returned to its cage. If no evidence of copulation was observed after the 14 day mating period, the female was separated from the male and the mating phase was concluded. At the termination of the study periods, all surviving F0 males and females were euthanized in a

	<p>humane fashion and subjected to gross necropsy examination. The F0 males were euthanized after approximately seven weeks of treatment. F0 females that underwent parturition were euthanized on lactation day 4. F0 females that failed to deliver were euthanized 25 days after evidence of mating. F0 females with no evidence of mating were euthanized 25 days after completion of the mating period. The necropsy examination on F0 males and females included evaluation of the external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. Uterine contents were examined and the number of implantation sites and number of corpora lutea (per ovary) were recorded. Uteri with no macroscopic evidence of implantation were opened and placed in 10% aqueous ammonium sulfide solution for detection of early embryo lethality. Testes of all F0 males were preserved in Bouin's fixative. Gross lesions, ovaries, prostate, epididymides, seminal vesicles uterus and vagina from all F0 animals were preserved in formalin for future histopathological examination. Statistical analysis was performed using ANOVA with Dunnett's post-hoc test, Chi-square test, and Mann-Whitney U test where appropriate for each specific endpoint. Analytical chemistry results indicated that the test material was homogenous and stable at the concentrations used in the study. <b>Subchronic effects:</b> Oral administration of the test material at doses of 100, 500 and 1000 mg/kg/day had no effect on F0 survival, and there were no remarkable clinical observations noted during the study. <b>Reproductive Indices, Precoital Intervals and Gestation Lengths:</b> There were no statistically significant or toxicologically meaningful differences in copulation or fertility indices among the groups. The copulation index was 100% in the control and the 1000 mg/kg/day groups, and 91.7% in the 100 and 500 mg/kg/day groups. The fertility index was 100% in the control, 100 and 1000 mg/kg/day groups, and 81.8% in the 500 mg/kg/day. No statistically significant differences were observed in group mean precoital intervals or gestation lengths. Mean precoital intervals in the control, 100, 500 and 1000 mg/kg/day groups were 1.5, 2.8, 3.5 and 2.6 days, respectively. Mean gestation lengths in the control, 100, 500 and 1000 mg/kg/day groups were 22.1, 22.0, 21.8 and 22.1 days, respectively. <b>F0 Gross Necropsy Observations:</b> Gross necropsy finding were generally unremarkable. When anomalies were observed, they were of low incidence, randomly distributed among the groups, and were not considered to be treatment related. <b>F0 Organ Weights:</b></p>
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	<p>There were no statistically significant or toxicologically meaningful differences in absolute or relative testes and epididymides weights between the control and test material-treated groups. <b>F0 Histopathology:</b> Histopathological examination of the testes, ovaries and epididymides from control and high-dose rats did not reveal any test material-related microscopic changes. <b>F0 Implantation and Pre/Post-Implantation Loss:</b> There were no statistically significant or toxicologically meaningful differences between control and test material-treated groups with respect to corpora lutea counts, implantation scar counts, mean number of live pups, or pre- or post-implantation loss. <b>F1 Pup Viability:</b> There were no toxicologically meaningful differences with respect to F1 pup viability, number of litters in each group with live offspring, mean live litter size or pup sex ratios. The incidence of dead pups on lactation day 0 was slightly higher (statistically significant) in the 500 mg/kg/day group. This difference was not considered toxicologically significant since a dose-response pattern was not demonstrated (i.e., the incidence of dead pups was comparable between the vehicle control and the 1000 mg/kg/day groups). <b>F1 Pup Observations during Lactation:</b> F1 pup observations during lactation were generally unremarkable with any individual findings randomly distributed among the groups and without dose-responsive pattern. <b>F1 Pup Body Weights during Lactation:</b> There were no statistically significant or toxicologically meaningful differences in F1 pup body weights during lactation. <b>F1 Pup Gross Necropsy Observations:</b> Among the F1 pups found dead or euthanized as scheduled on lactation day 4, gross necropsy did not reveal any findings which would indicate a relationship to treatment with the test material. The most notable gross observation in pups found dead on lactation day 0 consisted of atelectasis and absence of milk in the stomach, indicating that these pups were likely stillborn. Other necropsy findings tended to be of low incidence and randomly distributed among the groups. There were no indications of treatment-related developmental effects.</p>
<b><u>Conclusion</u></b>	The Study Director assigned a dosage level of 1000 mg/kg/day as the no observed effect level (NOEL) for this reproduction and developmental screening study in rats.
<b><u>Reference</u></b>	An oral (gavage) reproduction/developmental toxicity screening study in Sprague-Dawley rats with OS#132086. Springborn Laboratories Inc., 3/15/00.
<b><u>Data Quality:</u></b>	Valid without restriction – (Klimisch Code 1)

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